Mechanisms of Action of OPC-28326, a Selective Hindlimb Vasodilator

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ABSTRACT

The unique cardiovascular profile of OPC-28326 [4-(N-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionylaminobenzoyl)piperidine hydrochloride monohydrate] provides insight into basic mechanisms of this new drug as determined by experiments in dogs and rats. In anesthetized open-chest dogs, an i.v. administration of a low dose (0.3 and 1.0 µg/kg) of OPC-28326 selectively increased femoral artery blood flow with only minimal action on systemic blood pressure, heart rate and coronary, carotid, vertebral, renal, and mesenteric blood flows. Biochemical study suggests that OPC-28326 had no effect on phosphodiesterase-3 and -5. OPC-28326 dose-dependently inhibited phenylephrine-induced increases in blood pressure in spinally anesthetized dogs. The potency of OPC-28326 was, however, about 180 times lower than that of prazosin. Although binding studies have revealed an affinity of OPC-28326 to serotonin 5-HT2 receptors, the drug is without effect, except at very high concentrations, on serotonin-induced contraction in an isolated canine femoral artery preparation. The potency of OPC-28326 on the increase in femoral artery blood flow was about 14 times higher than that of prazosin but was at about the same level as that obtained with yohimbine in canine autoperfused femoral artery preparations. In perfused rat hindlimb preparations, OPC-28326 inhibited the decrease in perfusion flow induced by brimonidine, a selective α2-adrenoceptor agonist. The potency of OPC-28326 was at least 10 times less than that of yohimbine. Taken together, the results show that at low doses, OPC-28326 selectively exerts a potent vasodilating effect on the femoral arterial bed, in part due to an α2-adrenoceptor-blocking activity.

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ABBREVIATIONS: FBF; femoral artery blood flow; BP, blood pressure; SR, sinoatrial rate; ASA, anterior septal artery; OPC-28326, 4-(N-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionylaminobenzoyl)piperidine hydrochloride monohydrate; CF, contractile force; POAD, peripheral occlusive arterial disease; CBF, coronary artery blood flow; HR, heart rate; VBF, vertebral artery blood flow; CaBF, carotid artery blood flow; RBF, renal artery blood flow; MBF, mesenteric artery blood flow; 5-HT, 5-hydroxytryptamine (serotonin).
and TA-993, for example, increased blood flow to other organs, such as the brain (Kushiku et al., 1989; Kaburaki et al., 1998). Vintoperol decreased systemic blood pressure (Szombathelyi et al., 1991) at the same dose that increased FBF. A drug with a high selectivity for the femoral arterial bed would be of potential importance in the treatment of peripheral arterial insufficiency.

The purpose of the present study was to elucidate information on the mechanism of action of OPC-28326 using a variety of techniques in dogs and rats.

Materials and Methods

Mongrel dogs of either sex and Sprague-Dawley male rats (SLC, Shizuoka, Japan) were housed during the experiment in an air-conditioned (temperature-, humidity-, and light-controlled) animal room. All experiments were performed under the regulations of the Guidelines for Animal Experimentation (Otsuka Pharmaceutical Co., Ltd.).

Dogs weighing 8 to 23 kg were anesthetized with 30 mg/kg pentobarbital sodium i.v., and rats weighing 270 to 400 g were anesthetized with 50 mg/kg pentobarbital sodium i.p. All measurements were made with a thermal pen recorder (Recti-Horiz 8K; NEC Medical Systems, Tokyo, Japan).

Cardiovascular Effects of OPC-28326 in Anesthetized Open-Chest Dogs

After induction of anesthesia, an endotracheal cannula was inserted and connected to a respirator (model SN-480-3; Shinano, Tokyo, Japan) for ventilation with room air at a tidal volume of 20 ml/kg at a rate of 18 breaths/min. To maintain a constant level of anesthesia, the dogs received continuous pentobarbital sodium (4 mg/kg/h). A thoracotomy was performed at the fifth left intercostal space, and the heart was suspended in a cardiac cradle. An arch strain gauge (model TH-602T; Nihon-Kohden, Tokyo, Japan) was sutured to the left ventricular free wall for measurement of myocardial contractile force (CF). A polyethylene tube was placed in the left femoral artery and connected to a pressure transducer (model MPU-0.5) for measurement of BP. Heart rate (HR) was measured with a cardiac tachometer (model 1321; NEC Medical Systems) triggered by BP wave pulses.

Blood flow was measured with an electromagnetic flowmeter (model MFV-2100; Nihon-Kohden) and a pulsed Doppler flow system (MFV-2100; Crystal Biotech). Changes in frequency (df [kHz]) were converted into blood flow (milliliters per minute) using the following equation:

\[
\text{Blood flow (ml/min)} = 1.25 \times df \times D^2
\]

where D is the probe diameter (mm).

Electromagnetic flowmeter probes were placed on the right femoral artery to measure FBF. Vertebral artery blood flow (VBF) was measured with the same flowmeter probe placed on the artery after thoracotomy at the third left intercostal space. Carotid artery blood flow (CaBF) and CBF were measured with a Doppler flowmeter probe placed at the left side and at the origin of the left circumflex coronary artery, respectively. Renal artery blood flow (RBF) and mesenteric artery blood flow (MBF) were measured with a Doppler flowmeter probe placed on the arteries after abdominal incision. OPC-28326 was injected via the femoral vein.

When all parameters had stabilized, drug administration was started. Single doses of 0.1 to 30 \( \mu \text{g/kg} \) OPC-28326 were administered. When the parameters returned to pretreatment levels after the administration of a dose, another dose was administered.

In another experiment, the effects of OPC-28326 on BP and FBF were compared with those of prazosin using open-chest dogs. In this experiment, systolic and diastolic BPs and FBF were measured using a pressure transducer (model MPU-0.5) and electromagnetic flowmeter (model MFV-2100), respectively. The changes in each parameter after the i.v. administration of OPC-28326 (0.1–30 \( \mu \text{g/kg} \)) or prazosin (0.1–100 \( \mu \text{g/kg} \)), a selective \(\alpha_1\)-adrenoceptor blocker (Cambridge et al., 1977), were recorded.

\(\alpha_1\)-Adrenoceptor-Blocking Property of OPC-28326 in Spinally Anesthetized Dogs

After the induction of anesthesia, an endotracheal cannula was inserted and connected to a respirator (model SN-480-3) for ventilation with room air at a tidal volume of 20 ml/kg at a rate of 18 breaths/min. The left and right vagus nerves were cut, 1.0 mg/kg each of atropine and nadolol were injected intravenously, and 0.6 mg/kg dibucaine was injected into the cisterna magna at the level of the first segment of the cervical cord. A polyethylene tube was placed in the left femoral artery and connected to a pressure transducer (model MPU-0.5) for measurement of BP. Drugs were injected via a catheter placed in the left femoral vein. When BP had stabilized, the pressor response to i.v. administration of phenylephrine (10 \( \mu \text{g/kg} \)) was determined. In a preliminary study, the pressor response of phenylephrine was unchanged when repeatedly determined (seven determinations). Dogs were pretreated with increasing doses of OPC-28326 (1–1000 \( \mu \text{g/kg} \)) and the pressor responses were continuously recorded. In the case of prazosin, it was confirmed that the solvent (10% \( \text{N,N-dimethylformamide} \)), administered at a volume equal to that of 30 \( \mu \text{g/kg} \) drug, had no effect on the pressor response to phenylephrine. Prazosin was administered in a similar manner at 0.1 to 30 \( \mu \text{g/kg} \), and the pressor response was recorded. The pressor responses induced by phenylephrine were expressed as percentages of those recorded before the first dose of OPC-28326 or prazosin was given.

Effects of OPC-28326 on FBF in Autoperfused Canine Femoral Artery Preparations

Dogs were injected with 700 U/kg heparin sodium i.v. after the induction of anesthesia. The right femoral artery was perfused at 90 ml/min with the animal’s own blood from the carotid artery using a peristaltic pump (model 1219; Harvard Apparatus, Holliston, MA). A Starling pneumatic resistor was placed in parallel with the perfusion circuit to maintain the perfusion pressure at about 100 mm Hg. The blood flowing through the resistor was returned to the left femoral vein. Throughout the experiments, the animals were mechanically ventilated with room air via an endotracheal cannula connected to a respirator (model SN-480-3) using a tidal volume of 20 ml/kg at a rate of 18 breaths/min. The dogs were infused with pentobarbital sodium at 4 mg/kg/h to maintain anesthesia and with heparin sodium at 100 U/kg/h to prevent blood coagulation. FBF was measured with an electromagnetic flowmeter (MFV-2100) placed in the perfusion circuit. Drugs were administered to the femoral artery via a perfusion circuit using a microsyringe.

Cardiovascular Effects of OPC-28326 in Isolated, Blood-Perfused Canine Heart Preparations

Anesthetized dogs were injected with 500 U/kg heparin sodium i.v. and then exsanguinated. The heart was isolated, immersed in cooled lactate Ringer’s solution, and used for various isolated heart experiments. These preparations were kept in liquid paraffin at 37°C in a glass container and perfused with arterial blood from the carotid
artery of blood donor dogs through an arterial cannula using a peristaltic pump (model 1210; Harvard Apparatus). A Starling pneumatic resistor was placed in parallel with the perfusion circuit to maintain a constant perfusion pressure. The venous blood from the preparations and the blood passing through the resistor were collected in a blood reservoir and returned to the left jugular vein of the donor dog. The donor dogs were injected with 500 U/kg heparin sodium i.v. after the induction of anesthesia. Throughout the experiment, the dogs were ventilated and maintained with heparin and anesthesia as described above.

Sinoatrial node preparations, consisting of the right atrium, were prepared according to the method of Kubota and Hashimoto (1973). A cannula was inserted into the right coronary artery for perfusion at a constant pressure of about 100 mm Hg. Preparations were preloaded with a load of 1 g and stimulated with rectangular pulses (voltage, 1.2 times the threshold voltage) triggered by right atrial contraction. The right atrium preparations were reloaded with a load of 2 g. Drugs were administered using a microsyringe via a catheter connected to the right coronary artery.

Papillary muscle preparations were prepared from the ventricular septum and anterior chamber wall according to the method of Endoh and Hashimoto (1970). A cannula was inserted into the anterior septal artery (ASA) for perfusion at a constant pressure of about 100 mm Hg. Preparations were reloaded with a load of 1 g and stimulated with rectangular pulses (voltage, 1.2 times the threshold voltage; duration, 5 ms; frequency, 120 stimuli/min) generated by an electric stimulator (type 2907; NEC Medical Systems) triggered by right atrial contraction. The right atrium preparations were reloaded with a load of 2 g. Drugs were administered using a microsyringe via a catheter connected to the right coronary artery.

ASA was measured as an index of CBF. The drugs were administered using a microsyringe via a catheter connected to the ASA.

In the studies mentioned above, OPC-28326 at single doses of 0.1 to 100 nmol was administered when the pretreatment values of all the parameters had stabilized. When parameters returned to pretreatment levels after the administration of a single dose of the drug, another single dose was administered. To compare the potency in increasing FBF in autoperfused canine femoral artery preparations, OPC-28326 (0.1–100 nmol), prazosin (1–300 nmol), and yohimbine (1–300 nmol), a selective α2-adrenoceptor blocker (Shepperton, 1981), were administered via the femoral artery.

Receptor and Enzyme Studies

The affinities of OPC-28326 to various receptors and its actions on enzymes were examined at Panlabs Taiwan, Ltd. (Taipei, Taiwan, Republic of China). For each radioligand binding assay, target tissue, cell line, or recombinant receptor and radioligand were used as listed in Table 1. The specific binding was determined as percent assay. Enzyme inhibition were determined as percent inhibition of conversion of substrate or phosphorylation of substrate. The substrates used and the reactions observed were listed in Table 1.

### Inhibitory Action of OPC-28326 against Contraction Induced by 5-Hydroxytryptamine (5-HT) and Phenylephrine in Isolated Femoral Artery Preparations

Dogs were injected with 500 U/kg heparin sodium i.v. after the induction of anesthesia and exsanguinated before isolation of the femoral artery. The blood vessel was immersed in 37°C Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl2, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25.0 mM NaHCO3, 11.0 mM glucose, and

### Table 1

Effect of 10 μM OPC-28326 on radioligand binding and on inhibition of enzyme activity

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Membrane Source</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine A₁</td>
<td>[3H]DPCPX</td>
<td>Rat whole brain</td>
<td>5</td>
</tr>
<tr>
<td>Adenosine A₂A₃</td>
<td>[3H]CGS-21680</td>
<td>Rat striatum</td>
<td>10</td>
</tr>
<tr>
<td>Adrenergic α₁, nonselective</td>
<td>[3H]Prazosin</td>
<td>Rat whole brain</td>
<td>33</td>
</tr>
<tr>
<td>Adrenergic α₂, nonselective</td>
<td>[3H]Rauwolscine</td>
<td>Rat cerebral cortex</td>
<td>95</td>
</tr>
<tr>
<td>Adrenergic β₁</td>
<td>[3H]CGP-12177</td>
<td>Human recombinant</td>
<td>10</td>
</tr>
<tr>
<td>Adrenergic β₂</td>
<td>[3H]CGP-12177</td>
<td>Human recombinant</td>
<td>–1</td>
</tr>
<tr>
<td>Adrenergic β₃</td>
<td>[3H]Iodocyanopindol</td>
<td>Human recombinant</td>
<td>12</td>
</tr>
<tr>
<td>Angiotensin AT₁</td>
<td>[3H]Losartan</td>
<td>Rabbit adrenal gland</td>
<td>–9</td>
</tr>
<tr>
<td>Atrial natriuretic factor</td>
<td>111I-Labeled ANF</td>
<td>Guinea pig adrenal gland</td>
<td>14</td>
</tr>
<tr>
<td>Bradykinin B</td>
<td>[3H]des-Arg₁₁-kallidin</td>
<td>Human HS 729 cell</td>
<td>20</td>
</tr>
<tr>
<td>Bradykinin B</td>
<td>[3H]Bradykinin</td>
<td>Guinea pig ileum</td>
<td>–5</td>
</tr>
<tr>
<td>Ca²⁺ channel (L)</td>
<td>[3H]Dilitazem</td>
<td>Rat cerebral cortex</td>
<td>19</td>
</tr>
<tr>
<td>Dopamine D₁</td>
<td>[3H]SCH23390</td>
<td>Human recombinant</td>
<td>19</td>
</tr>
<tr>
<td>Endothelin ETA₂</td>
<td>125I-Labeled endothelin</td>
<td>Human recombinant</td>
<td>19</td>
</tr>
<tr>
<td>Endothelin ETA₃</td>
<td>125I-Labeled endothelin-1</td>
<td>Human recombinant</td>
<td>19</td>
</tr>
<tr>
<td>Histamine H₃, peripheral</td>
<td>[3H]Pyrilamine</td>
<td>Guinea pig lung</td>
<td>5</td>
</tr>
<tr>
<td>Histamine H₂</td>
<td>[3H]I-NH₂-potentidinetine</td>
<td>Guinea pig striatum</td>
<td>–6</td>
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<tr>
<td>Muscarinic, nonselective</td>
<td>[3H]QNB</td>
<td>Rat cortex</td>
<td>19</td>
</tr>
<tr>
<td>Neuropeptide Y₁</td>
<td>125I-Labeled neuropeptide Y</td>
<td>Human SK-N-MC cell</td>
<td>–11</td>
</tr>
<tr>
<td>Neuropeptide Y₂, Y₃</td>
<td>125I-Labeled neuropeptide Y</td>
<td>Rabbit kidney medulla</td>
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</tr>
<tr>
<td>Opiate, nonselective</td>
<td>[3H]Naloxone</td>
<td>Rat whole brain</td>
<td>3</td>
</tr>
<tr>
<td>Platelet-activating factor</td>
<td>[3H]PAF</td>
<td>Rabbit platelet</td>
<td>–13</td>
</tr>
<tr>
<td>Purinergic P₂X₁</td>
<td>[3H]Adenosine-β-me-ATP</td>
<td>Rabbit urinary bladder</td>
<td>23</td>
</tr>
<tr>
<td>Serotonin 5-HT₁</td>
<td>[3H]Ketanserin</td>
<td>Rat whole brain</td>
<td>34</td>
</tr>
<tr>
<td>Thromboxane A₂</td>
<td>[3H]SQ-29548</td>
<td>Rabbit platelet</td>
<td>12</td>
</tr>
<tr>
<td>Vasoactive intestine peptide VIP₁</td>
<td>125I-Labeled VIP</td>
<td>Guinea pig lung</td>
<td>–12</td>
</tr>
<tr>
<td>Vasoressin V₁</td>
<td>[3H]Arg-Vasopressin</td>
<td>Rat liver</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate, Reaction</th>
<th>Source</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO synthase, constitutive</td>
<td>[3H]Arginine → [3H]citrulline + NO</td>
<td>Rat celebellum</td>
<td>–8</td>
</tr>
<tr>
<td>Phosphodiesterase-III</td>
<td>[3H]cAMP → [3H]AMP (→ [3H]adenosine)</td>
<td>Human platelet</td>
<td>1</td>
</tr>
<tr>
<td>Protein kinase C, mixture of α, β, and γ</td>
<td>Histone H₁ (HH) + [γ-32P]ATP → [γ-32P]HH + ADP</td>
<td>Rat brain</td>
<td>–19</td>
</tr>
</tbody>
</table>
0.3 mM EDTA-Na$_2$ gassed with 95% O$_2$/5% CO$_2$, and stripped of connective and adipose tissues. The vessel was then cut into rings measuring 4 to 5 mm in length. Endothelial cells were carefully removed with forceps. The preparations were suspended vertically in an organ bath containing 20 ml of Krebs-Henseleit solution. The upper ends were connected to a force displacement transducer (UL-20GR; Minebea, Nagano, Japan). Initially, the vessel preparation was repeatedly contracted with 40 or 60 mM KCl. After the responses had stabilized, contraction was initiated with 5-HT (3 × 10$^{-7}$ M) or phenylephrine (3 × 10$^{-8}$ M). Concentrations were used that induced about 80% of maximum contraction. When contraction stabilized, OPC-28326 was cumulatively added to the organ bath at concentrations of 10$^{-8}$ to 10$^{-4}$ M. The vasorelaxing activities of OPC-28326 were expressed as percentages of contraction before treatment with the drug.

**α$_2$-Adrenoceptor-Blocking Property of OPC-28326 in Rat Perfused Hindquarters**

Rat perfused hindquarters were prepared according to a modification of the method of van Meel et al. (1985). Male Sprague-Dawley rats were treated with reserpine (5 mg/kg i.p.) and anesthetized with pentobarbital sodium 24 to 32 h later. The right hindquarter was perfused with modified Tyrode’s solution containing OPC-28326 (10$^{-8}$ to 10$^{-6}$ M) and 10$^{-7}$ M yohimbine or vehicle (distilled water) at constant pressure (55 cm H$_2$O) through the abdominal aorta. The rats were sacrificed by exsanguination, and the vena cava caudalis was cut to secure the outflow of perfusate. After stabilization, 1 ng to 30 µg of brimonidine, a selective α$_2$-adrenoceptor agonist (Guimaraes and Nunes, 1990; Thomas et al., 1994), was added to the perfusate in a volume of 10 µl. Changes in the perfusion flow rate of the hindquarter were expressed as percentages of the flow rate before the first dose of brimonidine.

Modified Tyrode’s solution was composed of 136.8 mM NaCl, 2.68 mM KCl, 0.26 mM MgCl$_2$, 0.42 mM NaH$_2$PO$_4$, 11.9 mM NaHCO$_3$, 1.8 mM CaCl$_2$, and 15 mM glucose, gassed with 95% O$_2$/5% CO$_2$, and maintained at room temperature (18–20°C).

**Drugs**

OPC-28326 (Otsuka Pharmaceutical Company, Tokyo, Japan), yohimbine (Sigma Chemical Co., St. Louis, MO), and phenylephrine (Wako Pure Chemical Industries, Osaka, Japan) were dissolved in distilled water and diluted with saline. Atropine (Nacalai Tesque, Kyoto, Japan) and dibucaine hydrochloride (Wako) were dissolved in distilled water. Nadolol (Sigma Chemical Co.) was dissolved in 0.5 N HCl. Prazosin (Sigma Chemical Co.) was dissolved in distilled water or 10% N,N-dimethylformamide (Wako) and diluted with distilled water. Brimonidine (Sigma) was dissolved in 20% N,N-dimethyl sulfoxide (Wako) and diluted with modified Tyrode’s solution.

**Statistical Analysis**

In all experiments, values are expressed as mean ± S.E., and differences were considered statistically significant at P < .05.

**Cardiovascular Effects of OPC-28326 in Anesthetized Open-Chest Dogs**

Differences between pretreatment and post-treatment values were analyzed by the paired t test (two-tailed) at each dose. Myocardial CF was expressed as a percentage of the value before the administration of 0.1 µg/kg OPC-28326, and the differences between normalized pretreatment values and post-treatment peak values were analyzed by Student’s t test (two-tailed) at each dose.

**Comparison of Effects of OPC-28326 and Prazosin on Cardiovascular System in Anesthetized Open-Chest Dogs**

Prazosin increased FBF dose-dependently. However, the increase was observed only at the doses that decreased BP (Fig. 5). On the other hand, OPC-28326 increased FBF dose-dependently, and the increase was statistically significant at all doses examined (Fig. 5). The systemic and diastolic
BPs were significantly decreased at higher doses (10 and 30 \( \mu g/kg \)). Thus, OPC-28326 increased FBF without any effect on systolic and diastolic BPs at lower doses.

**\( \alpha_1 \)-Adrenoceptor-Blocking Property of OPC-28326 in Spinally Anesthetized Dogs.** The baseline systolic BP values in the OPC-28326- and prazosin-treated groups before phenylephrine administration were 105 ± 7 and 107 ± 2 mm Hg, respectively. The increases in systolic BP after i.v. administration of phenylephrine (10 \( \mu g/kg \)) in the OPC-28326 and prazosin groups were 95 ± 11 and 108 ± 22 mm Hg, respectively. Baseline systolic BP and pressor responses induced by phenylephrine were not significantly different between the OPC-28326 and prazosin groups. OPC-28326 at doses of 1 to 10 \( \mu g/kg \) hardly affected systolic BP.

Effects of OPC-28326 on FBF in Canine Autoperfused Femoral Artery Preparations and on CBF, CF,
and SR in Blood-Perfused Canine Heart Preparations. FBF in six autoperfused femoral artery preparations was 27 ± 5 ml/min at a constant pressure of about 100 mm Hg. The basal tension developed in five papillary muscles stimulated at a rate of 120 stimuli/min was 6.8 ± 0.7 g, and the basal blood flow of ASA was 7.7 ± 1.0 ml/min at a constant pressure of about 100 mm Hg. In five sinoatrial node preparations, the basal SR was 98 ± 6 beats/min. Figure 7 shows the effects of OPC-28326 (0.1–100 nmol) on FBF in the constant-pressure autoperfused canine femoral artery preparations and on CBF, CF of papillary muscles, and SR in isolated, blood-perfused canine heart preparations. In this series of experiments, repeated intra-arterial administration of OPC-28326 did not affect the pretreatment values (data not shown). OPC-28326 dose-dependently increased FBF, with a 37% increase at a dose of 100 nmol in the constant-pressure autoperfused femoral artery preparations (Fig. 7). The increase in FBF was significant at doses of ≥30 nmol. OPC-28326 increased FBF at doses of 3 and 10 nmol in all five preparations, although these were not significant. In the isolated blood-perfused heart preparations, OPC-28326 at the doses tested had no effect on CBF, CF of papillary muscles, or SR (Fig. 7).

Effects of OPC-28326, Prazosin, and Yohimbine on FBF in Autoperfused Canine Femoral Artery Preparations. Baseline values of FBF in the OPC-28326-, prazosin-, and yohimbine-administered groups were 49.0 ± 8.5, 40.1 ± 9.3, and 51.0 ± 6.4 ml/min, respectively. There were no statistically significant differences among the drug-administered groups. OPC-28326, prazosin, and yohimbine increased FBF dose-dependently at doses of ≥1, ≥10, and ≥10 nmol, respectively (Fig. 8). Statistical analysis revealed parallelism between OPC-28326 (1–10 nmol), prazosin (10–100 nmol), and yohimbine (1–10 nmol). It was found from the line assay that yohimbine was approximately equipotent with OPC-28326 and that prazosin was 14 times less potent than OPC-28326.

Receptor and Enzyme Studies. The results of OPC-28326 on various receptors and enzymes are listed in Table 1. Even at 10 μM, the inhibitory effect of OPC-28326 on the enzyme activities listed and on the receptor binding did not reach 50% except for α2-adrenoceptors (Table 1). OPC-28326, at a concentration of 10 μM, inhibited the specific binding of [3H]rauwolscine (10 μM), a preferential α2-adrenoceptor blocker (Tanaka et al., 1978), by 95%. Further binding assay revealed that OPC-28326 inhibited the specific binding of [3H]rauwolscine to a rat brain preparation in a concentration-dependent manner (10 nM to 3 μM). The Ki value for α2-adrenoceptor was 337 ± 81 nM.

Inhibitory Action of OPC-28326 against Contraction Induced by 5-HT and Phenylephrine in Isolated Femoral Artery Preparations. Isolated canine femoral artery ring preparations were contracted with 3 × 10⁻⁷ M 5-HT or 3 × 10⁻⁶ M phenylephrine. Tension developed for each group was 3.66 ± 0.40 g and 4.75 ± 0.38 g, respectively. OPC-28326 relaxed phenylephrine-induced contraction concentration dependently. It did not, however, relax 5-HT-induced contraction until 10⁻⁵ M was reached (Fig. 9).

α2-Adrenoceptor-Blocking Property of OPC-28326 in Rat Perfused Hindquarters. In six perfused rat hindquarters, pretreatment values for perfusion of the modified Tyrode’s solution with OPC-28326 at concentrations of 10⁻⁸, 10⁻⁷, and 10⁻⁶ M, yohimbine at a concentration of 10⁻⁷ M, and its vehicle were 8.17 ± 0.44, 7.80 ± 0.52, 8.10 ± 0.48, 8.28 ± 0.5, and 7.88 ± 0.58 ml/min, respectively, at a constant pressure of about 55 cm H₂O. These values are not significantly different among the examined groups. When

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**Fig. 7.** Effects of intra-arterial OPC-28326 on FBF, CBF, SR, and papillary muscle CF in constant-pressure autoperfused canine femoral artery and isolated blood-perfused canine heart preparations. Each point is mean ± S.E. of five constant-pressure autoperfused canine femoral artery or six isolated blood-perfused canine heart preparations. *P < .05 compared with pretreatment values (paired t test).

**Fig. 8.** Effects of OPC-28326 (○), prazosin (□), and yohimbine (△) on FBF in constant-pressure autoperfused canine femoral artery preparations Changes in FBF after intra-arterial administration of the test drugs are shown. Each point is mean ± S.E. of four preparations.

**Fig. 9.** Effects of OPC-28326 on 5-HT- (●) and phenylephrine- (■) induced contractions in isolated canine femoral artery preparations. Percent values of tension (pretreatment values = 100%) are expressed as mean ± S.E. Each point consists of six preparations.
brimonidine (1 ng to 30 μg) was injected into the perfusate, perfusion flow was reduced in a concentration-dependent manner (Fig. 10). OPC-28326 at concentrations of 10⁻⁷ and 10⁻⁶ M inhibited flow reduction in a concentration-dependent manner (Fig. 10). Yohimbine, at a dose of 10⁻⁷ M, inhibited brimonidine-induced flow reduction significantly (Fig. 10). Exploratory analysis revealed that the inhibitory action of OPC-28326 at a concentration of 10⁻⁶ M was not significantly different from that of yohimbine at a concentration of 10⁻⁷ M, suggesting that OPC-28326 is at least 10 times less potent than yohimbine in inhibiting α₂-adrenoceptor activity.

**Discussion**

In the present study, OPC-28326 increased FBF dose-dependently with little change in systolic BP, diastolic BP, myocardial CF, and HR in anesthetized open-chest dogs. The blood flow to other organs, such as CBF, CaBF, VBF, RBF, and MBF, showed biphasic changes with an increase followed by a decrease, or vice versa, in the same preparations. The changes in these organ flows were smaller than that of FBF, especially at lower doses. OPC-28326 increased FBF dose-dependently in autoperfused canine femoral artery preparations when the drug was injected directly into femoral artery. The drug had no effect on HR, CF of papillary muscles, and CBF in isolated, blood-perfused canine heart preparations when injected directly into the coronary artery. From these results, we suggest that OPC-28326 is a dilator of femoral beds with little or no effect on other cardiovascular parameters.

A selective increase in blood flow to the hindlimb may be beneficial to patients with peripheral arterial insufficiency of the leg. There is, however, a controversy about the efficacy of vasodilators for this condition. Coffman (1979) reviewed past clinical trials and concluded that drugs that exerted vasodilation by affecting the sympathetic nervous system or via direct action were without value for patients with peripheral occlusive arterial disease (POAD). There are, however, some vasodilators that have been clinically proved to ameliorate POAD. Naftidrofuryl, an antagonist of 5-HT₂ receptors, for example, caused a vasodilation in the legs of dogs and humans (Barradell and Brogden, 1996). This agent increased pain-free walking distance in patients with intermittent claudication caused by POAD to a greater extent than placebo (Barradell and Brogden, 1996). Cilostazol, a phosphodiesterase-3 inhibitor, caused a flow increase during reactive hyperemia in the lower extremities of patients with arteriosclerosis obliterans (Yasuda et al., 1985), and this drug has beneficial effects in the treatment of intermittent claudication (Dawson et al., 1998). Buflomedil increased FBF dose-dependently in dogs (Vanhoutte, 1984) and improved POAD in humans (Clissold et al., 1987). Although these agents have other interesting effects, such as inhibition of platelet aggregation and/or favorable rheological actions (Clissold et al., 1987; Okuda et al., 1993; Barradell and Brogden, 1996), their common pharmacological effect, vasodilation, probably contributes in a major way to the amelioration of POAD. Roberts et al. (1987) reported that β-adrenoceptor blockers decreased pain-free and maximum walking distances on a treadmill at doses that reduced blood pressure in patients with hypertension complicated by intermittent claudication. Reduced systemic arterial pressure and reduction in cardiac output by a drug might exacerbate the reduction in perfusion pressure to the lower limb. Decreases in perfusion pressure can reduce lower limb blood flow as a consequence of compensatory adrenoceptor-mediated vasoconstriction in the collateral circulation and possibly in the stenotic vessel (Roberts et al., 1987). From these points of view, the selective and direct increase in FBF by OPC-28326 provides a good possibility for amelioration of POAD.

One of the possible mechanisms of vasodilation is the increase in cyclic nucleotides, such as cAMP and cGMP (Murray, 1990). Indeed, cilostazol and prazinap, an inhibitor of phosphodiesterase-5, which cause increases in cAMP and cGMP, respectively, induced vasodilation (Kamiya and Sakauchi, 1985; Trapani et al., 1991). OPC-28326, however, had almost no effect on these phosphodiesterases.

It has been reported that postsynaptic α-adrenoceptors exist in the hindlimb vasculature in dogs (Langer et al., 1981) and in rats (van Meel et al., 1983). Satoh et al. (1985) reported that an α₁-adrenoceptor antagonist preferentially increased FBF in anesthetized dogs. Binding studies indicate that OPC-28326 has a reasonable affinity to α₁-adrenoceptors. In this study, an inhibitory action of the phenylephrine-induced pressor response by OPC-28326 in spinally anesthetized dogs was, however, about 180 times less potent than that of prazosin, suggesting that OPC-28326 possesses α₁-adrenoceptor antagonistic activity, but its potency is very weak compared with prazosin. On the other hand, the potency in increasing FBF by OPC-28326 is 14 times more potent than that of prazosin. FBF was increased by OPC-28326 at doses that caused no effect on BP in open-chest dogs. Prazosin did increase FBF at doses that caused a decrease in BP. In other words, prazosin did not selectively increase FBF at any dose examined. Thus, we conclude that the α₁-adrenoceptor blockade per se is not the major mechanism of the FBF-increasing action of OPC-28326.

Receptor binding studies showed that OPC-28326 has an affinity to 5-HT₂ receptors. It has been reported that a bolus injection of 5-HT produced an increase in perfusion pressure in rat hindquarters (Verheyen et al., 1991). In collateralized femoral vascular beds, 5-HT-induced decreases in hindlimb flow was enhanced and the decrease was reduced by 5-HT₂ receptor antagonists (Orlandi et al., 1986; Verheyen et al.,

![Fig. 10. The effects of OPC-28326 (10⁻⁶ M, ●; 10⁻⁷ M, ▲; and 10⁻⁸ M, □), yohimbine (10⁻⁷ M, △), and vehicle (■) on decreased flow induced by brimonidine in rat perfused hindquarters. Each point is the mean ± S.E. of six preparations. **P < .01 OPC-28326 versus vehicle (two-way ANOVA). ††P < .01 yohimbine versus vehicle (two-way ANOVA). Analysis by two-way ANOVA revealed that the inhibitory action of OPC-28326 (10⁻⁶ M) was not significantly different from that of yohimbine (10⁻⁷ M).](image-url)
1991. These data suggest that the 5-HT₂ receptor greatly contributes to the modulation of the flow of femoral vascular beds. OPC-28326, however, did not relax 5-HT-induced contraction except in high doses in isolated canine femoral artery ring preparations. The inhibitory action of OPC-28326 against 5-HT-induced contraction was, if anything, much weaker than the decrease in FBF caused by clonidine and the inhibitory action against phenylephrine-induced contraction (α₁-adrenoceptor action). The possibility of involvement of 5-HT₂ receptor antagonist action to the increase in the FBF, however, cannot be completely discarded because there may be a difference in sensitivity between the femoral artery and the resistance vessels in the femoral vascular bed.

Clonidine, a preferential α₁-adrenoceptor agonist, decreased FBF with high potency in anesthetized dogs (Horn et al., 1982). The decrease in FBF caused by clonidine was inhibited by yohimbine. This suggests that the α₁-adrenoceptor may, at least in part, contribute to the contractile regulation of the femoral vascular bed (Horn et al., 1982). In the latter study, yohimbine increased FBF in autoperfused canine femoral artery preparations and inhibited the perfusion flow decrease in the hindlimb induced by brimonidine. These data suggest that α₁-adrenoceptor antagonists may increase FBF. OPC-28326 increased FBF in autoperfused canine femoral artery preparations. Furthermore, OPC-28326 inhibited brimonidine-induced decrease in perfusion flow in rat perfused hindquarters. Binding studies revealed that OPC-28326 has a high affinity to 5-HT₁b receptors (Kᵢ = 337 ± 81 nM). Thus, α₁-adrenoceptor antagonistic action may be one of the important mechanisms of action of OPC-28326.

The inhibitory action of OPC-28326 against brimonidine-induced flow reduction was, however, at least 10 times less potent than that of yohimbine in rat perfused hindquarters. On the other hand, the FBF-increasing effect of OPC-28326 is almost as potent as that of yohimbine in autoperfused canine femoral artery preparations. Thus, it is clear that some unknown mechanisms may contribute to the increase in blood flow. Further studies are required to reveal the exact mechanisms of selective vasodilation.

In conclusion, low doses of OPC-28326 increased FBF in anesthetized open-chest dogs with little changes in BP, HR, CF, and blood flow in other arteries, such as coronary, carotid, vertebral, renal, and mesenteric arteries. We suggest that this new drug is a selective peripheral vasodilator and may be of clinical relevance in some peripheral vascular disorders.

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